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Distribution of Aflatoxin in Grade Sample Components of Farmers Stock Peanuts

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ABSTRACT

A two-part study was conducted to measure aflatoxin concentrations in individual grade components of farmers stock peanuts. Components were sound mature kernels (SMK), sound splits (SS), loose shelled kernels (LSK), other kernels (OK), and damaged kernels (DK). In Part 1, components of 151 official grade samples, averaging 1.8 kg, were analyzed for aflatoxin, whereas, in Part 2, analyses were conducted on components of 52 11.3-kg samples taken from 27 farmers stock lots. Results showed that the mean aflatoxin concentration for all samples in Part 1 was 89 ppb and that most of the toxin was associated with the OK, LSK, and DK components. Aflatoxin in the SMK + SS components averaged only 3 ppb, whereas aflatoxin in the OK, LSK, and DK averaged 247, 403, and 4107 ppb, respectively. Similar results were obtained in Part 2 with an overall average of 64 ppb and component averages of 4 ppb (SMK), 1 ppb (SS), 109 ppb (OK), 424 ppb (LSK), and 3188 ppb (DK). In many contaminated lots, the SMK and SS components, which comprised 85% of the total kernel weight, were minimally contaminated (1-2 ppb). Correlation coefficients between aflatoxin concentrations in total grade samples and the combined OK, LSK, and DK components suggest that analysis of just those components could provide a better indication of the aflatoxin risk associated with a lot than is provided by the visual *Aspergillus flavus* method used in the current grading system. In addition, separate storage of contaminated lots would allow reclamation of the noncontaminated SMKs during years when peanuts are in short supply.

Key Words: *Arachis hypogaea* L., *Aspergillus flavus*, *Aspergillus parasiticus*, farmers stock, groundnuts, HPLC.

Aflatoxins are hepatotoxic, carcinogenic metabolites of the fungi, *Aspergillus flavus* Link and *A. parasiticus* Speare (2). These fungi can invade and grow in various agricultural commodities, including peanut (*Arachis hypogaea* L.), resulting in their contamination with the aflatoxins. Preharvest aflatoxin contamination of peanuts occurs under conditions of heat and drought stress during the latter stages of the growing season; and, when such climatic conditions exist over a wide area of peanut-producing regions, significant contamination of the crop occurs (1, 5, 11, 14, 17).

The U.S. peanut industry uses an aflatoxin monitoring and control program whereby all lots of shelled peanuts destined for human consumption are analyzed for aflatoxin. To be considered acceptable for sale, the lot must test for less than 15 ppb of aflatoxin (13). However, the earliest steps in the aflatoxin control program are taken at the farmers stock level when peanuts are taken from the field to the first point of sale. A sample of peanuts from the farmers stock lot is visually examined for the presence of *A. flavus* (VAF method). If *A. flavus* is found on any peanut, the lot cannot be used for edible purposes and is designated as Segregation 3. If *A. flavus* is not found, the lot is accepted, designated as Segregation 1, and is usually combined with other accepted farmers stock lots in a bulk storage warehouse to await shelling. Peanuts are not analyzed for aflatoxin at this point, and significant aflatoxin contamination may be present even though no kernels with visible *A. flavus* are found.

During the normal farmers stock grading procedure, peanuts are divided into various components to determine the value of the lot. These components are sound mature kernels (SMK), sound splits (SS), other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DK). Earlier studies have shown that all peanuts in a farmers stock lot do not have the same risk of aflatoxin contamination (3, 4, 9, 10). Even in contaminated lots, most of the peanuts do not have aflatoxin. Rather, the toxin is usually associated with a relatively small number of highly contaminated kernels. This leads to extreme variability in aflatoxin analyses when multiple samples from a lot are analyzed (7, 15, 16).

In a study that compared the VAF method with enzyme-linked immunosorbent assay (ELISA) for identifying farmers stock grade samples contaminated with aflatoxin, Cole *et al.* (3) showed the aflatoxin distribution among components of 44 grade samples contaminated with >20 ppb of aflatoxin. Whereas that study demonstrated a trend in how aflatoxin was distributed among grade sample components, the emphasis of the paper was on the effectiveness of the VAF and ELISA methods, and no conclusions were presented with regard to the aflatoxin distribution data. The purpose of this paper is to present the complete aflatoxin distribution data from 151 farmers stock grade samples analyzed in the prior study (3) (Part 1) as well as the aflatoxin distribution in 27 additional farmers stock lots based on analysis of much larger (11.3 kg) samples (Part 2). A discussion of how the aflatoxin distribution in grade sample components could be utilized to better assess the aflatoxin risk associated with farmers stock lots also is presented.

Materials and Methods

Part 1. The Federal-State Inspection Service grade sample components were collected from 151 farmers stock lots at a southwest Georgia peanut buying point over a 6-d period during the 1986 peanut harvest season. The components of the grade samples consisted of SMK, SS, LSK, OK, and DK. The samples were brought to the laboratory and the entirety of each component (SMK and SS were combined) was extracted with 80:20 methanol/water (v/v) (2

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mL/g) in a Waring blender and analyzed for aflatoxin by high performance liquid chromatography (HPLC) (8). An aflatoxin value for each composite grade sample was calculated on the basis of the weight and aflatoxin concentration of each component of the sample.

Part 2. Twenty-seven loads of farmers stock peanuts were sampled by pneumatic probe at a peanut buying point in southwest Georgia during the 1986 harvest season. Two 11.3-kg (25 lb) samples were drawn from each of 25 loads, and one sample was drawn from each of the other two loads. The entirety of each sample was processed by inspectors of the Federal State Inspection Service. This process included removal of foreign material and LSK, shelling of the peanuts, and separation of shelled peanuts into normal grade components including SMK, SS, OK, and DK.

Each component was analyzed for aflatoxin as in Part 1. Because of its size, the SMK component was ground in a Dickens mill (6) and thoroughly blended in a small cement mixer for 20 min before removing two 200-g subsamples for analysis. Each of the other components (LSK, OK, DK, and SS) was analyzed in its entirety by extracting it with 80:20 methanol/water (v/v) (2 mL/g) in a Waring blender of the appropriate size (up to 3.8 L). Duplicate analyses were performed on each extract, and the reported results are the mean of the two analyses. The weighted composite aflatoxin concentration for each sample was calculated as in Part 1. Statistical analyses were conducted using the SigmaStat statistical software system (12).

Results

Part 1. The average weights of the grade sample components were SMK + SS, 237 g (68%); LSK, 77 g (22%); OK, 29 g (8%); and DK, 3 g (1%). The LSK percentage is disproportionately large because, in the grading procedure, the LSK are taken from approximately 1800 g of peanuts, whereas the other components come from only 500 g. Therefore, in computing the composite aflatoxin concentration for each sample (Table 1), the LSK weights were adjusted down by 72% to eliminate an unusually heavy weighting toward the LSK.

Results of aflatoxin analyses of the grade components and calculated composite sample aflatoxin values are presented in Table 1. Lots are grouped by segregation, and lots that tested negative for aflatoxin are grouped together on the first line within each segregation. Contaminated lots are ordered in increasing composite aflatoxin concentration for ease of readability.

Samples from the 107 Segregation 1 lots had a mean composite aflatoxin concentration of 52 ppb (ng/g). This compares with 39 samples from segregation three lots with a mean composite aflatoxin concentration of 200 ppb. The mean composite aflatoxin concentration for all samples was 89 ppb. The component with the least aflatoxin was the SMK + SS, which averaged 3 ppb over all lots. This compares with 247 ppb for the OK, 403 ppb for the LSK, and 4107 ppb for the DK. The DK are picked out of the SMK and SS components, and this tends to concentrate much of the aflatoxin in a small component.

The LSK, OK, and DK components each contributed from 31 to 33% of the total aflatoxin by weight (Fig. 1). The SMK and SS components, which made up about

Table 1. Aflatoxin in components of farmers stock grade samples and the calculated aflatoxin concentration (weight basis) in the composite samples.[a]

Lot #	Segregation	SMK + SS	OK	LSK	DK	Composite[b]
		ppb				
1-4	NG[c]					0
5	NG	0	1265	623	0	167
Seg. NG Mean:		0	253	126	0	34
6-81	1					0
82	1	0	0	8	NA[d]	1
83	1	0	0	10	0	1
84	1	0	0	5	1	1
85	1	1	0	1	0	
86	1	1	1	1	15	1
87	1	2	1	1	2	1
88	1	0	16	0	9	2
89	1	0	0	22	NA	2
90	1	1	0	41	NA	2
91	1	0	0	36	0	5
92	1	0	0	192	NA	8
93	1	0	0	114	0	9
94	1	0	0	240	199	14
95	1	19	0	0	NA	17
96	1	23	0	0	14	20
97	1	0	0	243	NA	22
98	1	29	10	NA	0	27
99	1	0	0	450	0	43
100	1	0	8	767	0	43
101	1	0	0	398	NA	44
102	1	0	469	0	1	53
103	1	0	859	1	6	62
104	1	0	734	9	0	80
105	1	0	1500	0	0	116
106	1	0	756	0	2	121
107	1	0	414	1378	0	124
108	1	6	0	1547	1	175
109	1	0	0	2338	19	218
110	1	0	1149	3449	2	359
111	1	3	3	5374	172	1078
112	1	9	4056	2158	298173	2891
Seg. 1 Mean:		1	94	177	3601	52
113-123	3					0
124	3	0	0	7	0	1
125	3	0	0	11	4	1
126	3	0	0	17	3	2
127	3	0	0	8	4091	6
128	3	0	0	403	0	19
129	3	0	144	302	0	25
130	3	0	9	212	948	28
131	3	0	18	408	3	34
132	3	0	296	228	1	35
133	3	1	1	403	2	43
134	3	0	31	363	1002	69
135	3	0	1665	13	0	89
136	3	0	982	1	0	90
137	3	0	9	20	5242	115
138	3	111	597	320	3	158
139	3	0	325	1323	5	163
140	3	7	0	3283	4767	227
141	3	0	704	2469	1935	380
142	3	0	3000	1540	3	398
143	3	0	1	2733	1234	429
144	3	111	208	53	19630	445
145	3	101	0	0	55900	587
146	3	0	6900	2210	956	639
147	3	2	3575	1879	NA	665
148	3	0	112	199	118686	668
149	3	0	7109	62	0	739

Table 1 (Continued)

Lot #	Segregation	SMK + SS	OK	LSK	DK	Composite[b]
		ppb				
150	3	0	5	14833	0	806
151	3	0	181	7697	16	935
Seg. 3 Mean:		9	663	1052	5643	200
Overall Mean:		3	247	403	4107	89

[a]A portion of the data presented in this table has been published previously (3).

[b]Aflatoxin concentrations for composite samples were calculated by dividing the total toxin weight (ng) from all components by the total kernel weight (g) of all components. LSK weights were mathematically reduced by 72% to compensate for the fact that LSK were taken from 1800 g while other components were taken from only 500 g.

[c]NG = Segregation not given.

[d]NA = Component missing or otherwise not available for analysis.

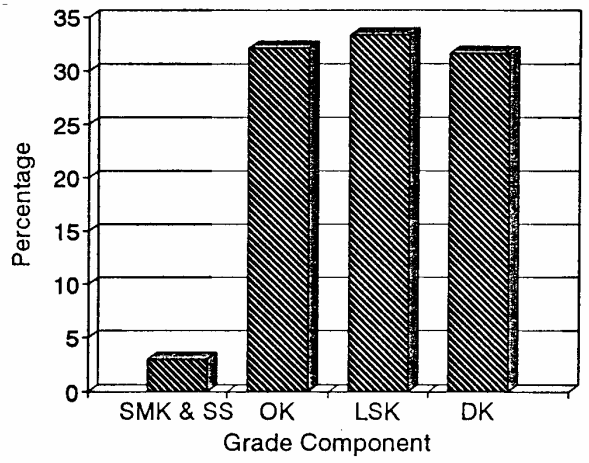

Fig. 1. Percentage of total aflatoxin contributed by each grade component in Part 1.

80% of the total kernel weight (using LSK adjusted weights), contributed only 3% of the total aflatoxin.

Part 2. Although all lots were graded as Segregation 1, most were contaminated with aflatoxin (Table 2). The average weights of the components were: SMK, 6532 g (79% of the total kernel weight); SS, 496 g (6%); OK, 776 g (9%); LSK, 419 g (5%); and DK, 75 g (1%). Overall, aflatoxin was lowest in the SS component, averaging 1 ppb. The average aflatoxin concentration of all SMK samples also was relatively low at 4 ppb. However, the average composite aflatoxin value was 64 ppb, reflective of the high concentrations found in many of the OK, LSK, and DK components.

The SMK and SS components contributed about 5% of the total aflatoxin by weight found in these samples (Fig. 2). The OK, LSK, and DK contributions were 22,

Table 2. Aflatoxin in components of 11.3-kg samples of farmers stock peanuts graded as segregation 1.

Lot #	SMK	SS	OK	LSK	DK	Composite[b]
	ppb					
356	0	0	0	0	0	0
356	0	0	0	0	0	0
359	0	0	0	0	0	0
359	0	0	0	2	0	0
23	0	0	0	0	1	0
23	0	0	1	0	0	0
85	0	1	1	1	1	0
85	1	1	1	2	1	
408	0	0	2	3	0	0
408	2	0	2	31		3
11	0	0	0	0	1	0
11	0	0	148	21	3	12
10	0	0	0	0	18	0
10	0	1	0	66	53675	84
414			0	15	0	1
414	1		1	2016		57
378	0		0	27	0	2
378	1	0	45	0	90	3
6	0	1	25	6	1	2
6	0	1	2	2	291	4
78	0	0	7	0	1497	2
78	1	1	89	0	0	8
92	0	0	1	48	0	4
92	0	1	2	2	4884	60
200	1	1	0	0	570	6
200	1	1	17	0	620	8
135	1	1	1	1	3405	6
135	1	0	1	14	5579	13
107	1	1	1	3	669	7
82	0	0	3	0	526	8
82	1	0	1	60	1183	20
46	1	0	0	1	1012	9
46	0	0	1	1	2560	24
428	0	1	2	3	1024	19
428	1	1	196	15	4653	64
417	2	0	5	50	1830	22
417	1	0	8	499	4183	62
2	0	0	256	726	1270	61
409	1	1	88	1175	841	66
409	26	1	222	1446	2781	95
72	2	1	253	1040	885	110
72	1		443	1286	4228	178
608	2	1	149	400	5448	110
608	107	1	269	1377	2464	237
3	0	1	191	813	2090	131
3	33	2	705	75	7067	211
190	6	2	359	481	4674	149
190	4	5	268	2792	10464	355
406	1	2	94	2409	2577	157
406	2	1	868	2133	1808	346
427	16	1	650	51	21090	303
427	2	1	288	2976	9823	328
Mean	4	1	109	424	3188	64

[a]Aflatoxin concentrations for composite samples were calculated by dividing the total toxin weight (ng) from all components by the total kernel weight (g) of all components.

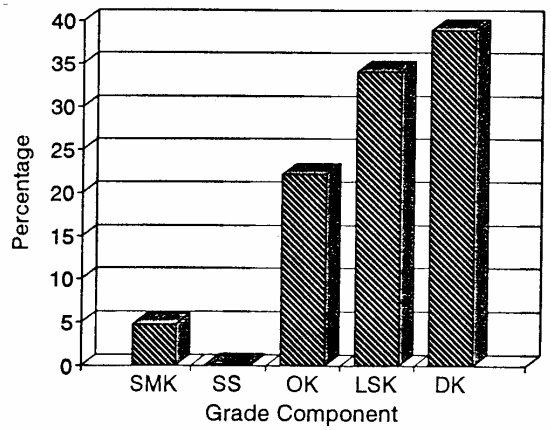

Fig. 2. Percentage of total aflatoxin contributed by each grade component in Part 2.

34, and 39%, respectively. Although the percentage of total aflatoxin found in the OK, LSK, and DK components was somewhat different from Part 1, there is a striking similarity in the total aflatoxin contributed by the more important SMK and SS components. Different buying points at different locations were used for Parts 1 and 2. However, in both, aflatoxin was concentrated in the poorer quality peanuts (OK, LSK, and DK). The OK are peanuts that fall through a 6.35-mm (16/64 in.) slotted screen. Similar peanuts in a shelling plant are used only for oil stock. LSK are generally of poorer quality than SMK, and during years with significant late season drought stress, they are usually highly contaminated with aflatoxin. In both parts of this study, there was an approximate 10-fold increase in aflatoxin from the SMK + SS to the OK and LSK components. This was followed by another 10-fold increase in the DK component (Tables 1 and 2). However, because the DK component usually makes up such a small percentage of the total weight, its overall contribution to total aflatoxin appears to be about the same as the OK and LSK.

Correlation coefficients between aflatoxin concentrations in the composite samples and various grade components and grade component combinations are presented in Table 3. For the purpose of this analysis each lot replicate in Part 2 was treated as a separate sample. In Part 1, the highest correlation (0.996) was between the OK + LSK + DK and composite aflatoxin. In Part 2, the highest correlation was between the OK + LSK and the composite. However, the OK + LSK + DK correlation increased from 0.793 to 0.951 when the second replicate sample from Lot #10 was removed from the data set. The unusually high aflatoxin concentration in the DK (53,675 ppb) produced a point in the regression analysis that might be considered an outlier.

Discussion

The high correlation between aflatoxin in the combined OK + LSK + DK components and the composite samples is not surprising since most of the aflatoxin was

Table 3. Correlation coefficients between aflatoxin concentrations in the total sample and in various grade components and grade component combinations.

Component	Total sample (SMK + SS + OK + LSK + DK)	
	Part 1	Part 2
SMK	0.182	0.384
OK	0.562	0.831
LSK	0.521	0.760
DK	0.842	0.354
OK + LSK	0.721	0.918
OK + DK	0.902	0.506
LSK + DK	0.934	0.806
OK + LSK + DK	0.996	0.793

contained in these components. In a data set in which the SMK fraction also was routinely contaminated, such a high correlation may or may not be the case. However, even in those instances, analysis of the OK + LSK + DK components should provide good information on the aflatoxin risk associated with such lots since the data indicate that they are the highest risk components.

The data presented in this study suggest that alternative methodology should be considered for characterizing the aflatoxin risk associated with farmers stock peanuts. Results showed that the high aflatoxin-risk components in farmers stock peanuts are the DK, LSK, and OK. Although these components offer the greatest chance for detecting aflatoxin, they make up a relatively small portion of the grade sample. In addition, the grade sample is never analyzed directly for aflatoxin. If a method could be implemented that allows collection of a larger portion of these high risk components combined with a chemical analysis of those components for aflatoxin, then many more lots that contain aflatoxin should be identified. When these components are negative for aflatoxin, the probability is great that the lot as a whole is negative also. Such lots can be shelled quickly and efficiently with minimal rejection of peanuts. Lots in which the high risk components contain aflatoxin could be segregated from noncontaminated lots and shelled with the knowledge that stringent sorting and possibly other measures would be necessary for them to meet aflatoxin specifications for shelled lots. It would be a mistake to make all such lots segregation 3 at the farmers stock level because in a year, such as 1990, when peanuts are in short supply, the SMK and SS components could be utilized for the edible market. In Part 2 of this study, the SMK and SS made up 85% of the total kernel weight and averaged about 5 ppb of aflatoxin. Whereas it is desirable to segregate contaminated lots from noncontaminated lots, it is uneconomical to condemn all contaminated lots to oil stock when the majority of the peanuts could be salvaged.

In conclusion, three major points are shown by this study. (a) In years that are conducive to preharvest aflatoxin contamination of peanuts, many contaminated farmers stock lots pass the grading process as segregation

l and are co-mingled in warehouses with noncontaminated lots. This results in great economic loss to the peanut industry. (b) The farmers stock components with the highest risk for aflatoxin contamination (DK, LSK, and OK) make up only about 15% of the total kernel weight. These could be concentrated from a larger grade sample than is currently taken and analyzed directly for aflatoxin, thus providing a more accurate measure of the aflatoxin risk associated with the farmers stock lot. (c) Proper segregation and storage of contaminated lots would allow for concentrated efforts in the milling of such lots in order to salvage the high quality SMK and SS components in years when peanuts are in short supply.

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